Effects of the Olfactory Environment and Nutrition on the Ability of Male Mediterranean Fruit Flies to Endure Starvation

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ABSTRACT The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is targeted for control using the sterile insect technique (SIT). For this technique to succeed, released males must be able to compete with wild males for copulations. Male success is mediated by survival in the field often in adverse conditions. Manipulation of the postteneral environment experienced by sterile males before release has been shown to affect male sexual success and survival. The objectives of this study were to determine how various diets, combined with exposure to volatiles containing α-copaene, affect the ability of male Mediterranean fruit flies (from a wild and two unisexual strains) to withstand starvation. Accordingly, we maintained males on one of eight regimes combing a diet of either sugar, sugar and protein, a protein pulse or apricot, with or without the aroma of the sexual stimulant α-copaene. The apricot diet was associated with the lowest ability to resist starvation. The sugar-only diet was associated with the highest ability to resist starvation by sterile males. Exposure to α-copaene, in combination with the apricot diet, had a significant negative effect on the ability of males (from all strains) to resist starvation relative to other regimes examined. We conclude that the holding regimes that elicit the best sexual performance from males paradoxically also hasten their demise, probably by initiating an irreversible metabolic cascade. The search for the optimal prerelease regime continues.

KEY WORDS sterile insect technique, *Ceratitis*, survival, nutrition, α-copaene

THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a cosmopolitan, polyphagous pest in fruit growing agroecosystems. Fruit growers around the world bear the economic burden of infestations (or threats of infestations) caused by this pest. Although malathion or spinosad sprays are still commonly used against fruit fly breakouts, the environmentally friendly sterile insect technique (SIT) is increasingly seen by local and international control agencies as the lynchpin of the integrated management of this pest (Hendrichs et al. 1995, 2002).

In SIT operations, large numbers of mass-reared, sterile males are released into the field, where they are expected to attract, court, and copulate females from the wild population. Although the SIT has been shown to be successful against the Mediterranean fruit fly, it is an industrial process that can be constantly improved and streamlined (Robinson et al. 2002). The objective of such improvements is to find cost effective ways to enhance the sexual performance of sterile males in the field.

In recent years, two complementary approaches aimed at postteneral males have been successful in improving sexual performance. One targets the olfactory environment, and the other, nutrition. Shelly and coworkers have shown that permeating the postteneral environment with volatiles containing α-copaene significantly improves subsequent sexual performance of the males (Shelly et al. 2003, and references therein). Similarly, Yuval and coworkers have shown repeatedly that inclusion of protein in the postteneral diet enhances the ability of males to copulate females and inhibit their receptivity to further copulations (reviews in Yuval and Hendrichs 2000, Yuval et al. 2002).

Although improving male performance is a worthwhile goal, one must be assured that the treatment affecting sexual performance does not negatively affect longevity, because shortening the life span of the released male may compromise the effectiveness of the SIT. The effect of protein nutrition on survival has been shown to be rather complex: protein fed sterile males are rapidly overcome by starvation and die after 24 h without nourishment, whereas most sugar-fed flies are still alive (Kaspi and Yuval 2000). Furthermore, starvation negatively affects the sexual performance of wild males within 24 h (Shelly and Kennelly 2003). However, if food is available in the release environment, protein fed sterile males are capable of finding it and live as long as males fed no protein before release, with the attendant sexual benefits (Maor et al. 2004).

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As we design the optimal prerelease environment for sterile males, we must therefore consider the effects of our manipulation on the survival of the flies in an environment that, for them, is relatively hostile. Accordingly, the objectives of this study were to determine how various diets, combined with exposure to volatiles containing α-copaene, affect the ability of male Mediterranean fruit flies (from one wild and two mass reared strains), to withstand starvation.

Materials and Methods

We used flies from three sources. Wild flies were first generation descendants of wild males and females collected in a fruit grove near Rehovot on the coastal plain of Israel and allowed to oviposit in the laboratory, as described by Kaspi et al. (2001). On the day of eclosion, the flies were segregated by sex.

Sterile males of the Vienna 7/Tol-2000 'genetic sexing' strain (henceforth SIT1) were obtained as pupae en route to the SIT release program in the Arava region of Israel. These flies are produced by the El Pino rearing facility in Guatemala, where they were irradiated (150 Gy), covered with Dayglo Orange fluorescent dust 2 d before emergence, and then sent by air to Israel.

Sterile males of a second genetic sexing strain Vienna 4/Tol-99 (henceforth SIT2) were obtained as pupae from the California Department of Food and Agriculture (CDFA) rearing facility in Hawaii, where they were similarly irradiated and dyed before transfer to the USDA laboratory in Manoa, Hawaii, where the experiments with this strain were conducted.

After eclosion, adult males were kept in 5-liter plastic containers at densities of 40 flies per container.

There were four diet regimes that combined with exposure to α-copaene, resulted in eight experimental treatments. The diet regimes were as follows:

1. Sugar. Sugar gel (18% sucrose; 0.915% agar; and 0.011% methylparaben, $C_8H_8O_3$). This is the prerelease diet provided to sterile males in SIT control operations, such as Eastern Mediterranean sterile medfly project, in southern Israel.
2. Sugar + protein. Males were provided with two petri dishes, one containing the sugar diet (as described in 1) and one containing the same diet with the addition of 9% protein hydrolysate. The rationale for this diet was that males would be able to select the optimal ratio of sugar and protein (Cangussu and Zucoloto 1995).
3. Sugar, protein day 2. Sugar gel was given on days 1 and 3. On day 2, the sugar gel was replaced with protein gel (in the same formulation as described above). The rationale for this diet was that a pulse of protein may provide the benefits associated with protein but not the negative effects (Kaspi and Yuval 2000; Maor 2004). This diet was not assayed in the experiments with the SIT2 strain in Hawaii.
4. Apricot. Dried apricot and ad libitum access to water. This diet was associated with the best sexual performance by sterile males (Maor 2004). We used commercially available dried apricots that were rinsed and split before presentation to the flies.

α-Copaene was provided by exposing flies, from the day of eclosion, to the volatiles of ginger root oil (GRO), a natural product which contains 0.4% α-copaene. We applied 250 μl of ginger root oil (Citrus and Allied Essences, Lake Success, NY) to an Eppendorf tube with three small holes, to allow constant release of the volatiles.

Males were exposed to the various diets and olfactory treatments continuously from the day of eclosion until they were 4 d old (the age at which sterile males are released). In Rehovot, flies were held in natural illumination (photoperiod of ≈14:10 [L:D] h), 24–29°C, and 50–70% RH. In Manoa, conditions were photoperiod of 12:12 (L:D) h, 22–27°C, and 65–90% RH. On the fourth day postemergence, the treatments (dish or dishes containing food and tubes containing GRO) were removed, cages were wiped clean, and 24–26 flies were left in each cage. After 24 h, the cages were observed twice a day, at 0800 and 2000 hours, until all the flies died.

Statistical Analysis. Each experiment was repeated three times. To evaluate the effect of diet and olfactory environment and strain on survival we used parametric survival analysis (log-rank test) in the JMP statistical package.

Results

The apricot diet was associated with the lowest ability to endure starvation (Figs. 1–3). Significantly, males from all three strains examined (wild, SIT1, and SIT2), fed on the apricot diet had a lower resistance to starvation than males fed other diets (Table 1). Furthermore, exposure to GRO, in combination with the apricot diet, had an additional significant negative effect on the ability of males (from all strains) to resist starvation (wild, log-rank test: $\chi^2 = 47.61$, df = 1, $P < 0.0001$; SIT1, log-rank test: $\chi^2 = 14.86$, df = 1, $P < 0.0001$; SIT2, log-rank test: $\chi^2 = 12.22$, df = 1, $P = 0.0005$). Thus, the apricot diet combined with GRO resulted in lowest ability to resist starvation, followed by the apricot diet without GRO volatiles.

The sugar diet was associated with the highest survival of sterile males. Males from both mass reared strains fed on the sugar diet (alone or in combination with exposure to GRO) were significantly likelier to survive longer, after food was removed, than males fed all other diets (Figs. 2 and 3; Table 1). In the groups of wild males, sugar-fed males survived longer than males fed sugar + protein (log-rank test: $\chi^2 = 10.64$, df = 1, $P = 0.001$), but not significantly longer than males fed one protein meal on day 2 after emergence (log-rank test: $\chi^2 = 3.03$, df = 1, $P = 0.082$). The combination of GRO exposure with the sugar diet, compared with sugar diet alone, did not significantly affect survival (wild, log-rank test: $\chi^2 = 0.24$, df = 1, $P = 0.624$; SIT1, log-rank test: $\chi^2 = 0.71$, df = 1, $P =$

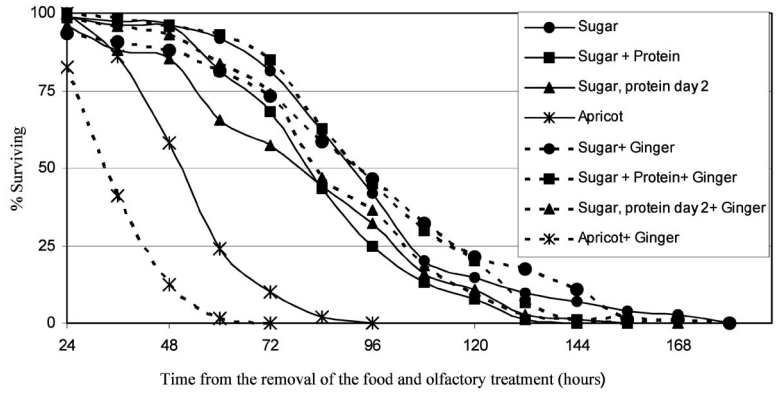

Fig. 1. Survival duration of wild male Mediterranean fruit flies maintained on different olfactory and dietary regimes after removal of food. Pooled results from three replicates.

0.398; and SIT2, log-rank test: $\chi^2 = 1.70$, df = 1, $P = 0.192$) (Table 1).

The sugar + protein diet, compared with the sugar diet, was significantly associated with lower survival in all three strains (Table 1). When GRO exposure was combined with this diet, results varied according to strain. In the SIT1 strain, the addition of GRO had no additional effect on survival (log-rank test: $\chi^2 = 0.004$, df = 1, $P = 0.948$). However, exposure to GRO had a significant negative effect in combination with sugar + protein diet on the ability of males from the SIT2 strain to resist starvation (log-rank test: $\chi^2 = 3.88$, df = 1, $P = 0.048$). Conversely, in wild males, exposure to GRO had a significant positive effect when combined with the sugar + protein diet (log-rank test $\chi^2 = 6.77$, df = 1, $P = 0.0093$).

The sugar, protein day 2 diet was only assayed in the wild and SIT1 strains. As mentioned above, the survival of wild males fed on this diet was not significantly different from that of those fed the sugar diet (Table 1). However, the survival of sterile males fed this protein pulse was significantly lower than that of sterile males fed only on the sugar (log-rank test: $\chi^2 = 10.15$, df = 1, $P = 0.001$). When combined with exposure to GRO volatiles, no further effects on survival were observed (Table 1; Figs. 1 and 2).

To determine whether the ability to resist starvation is associated with the strains we worked with, we compared the pooled survival rates of the three strains. Significantly, sterile males have a lower ability to resist starvation compared with wild males (wild-SIT1, log-rank test: $\chi^2 = 869.70$, df = 1, $P < 0.0001$; wild-SIT2, log-rank test: $\chi^2 = 280.02$, df = 1, $P < 0.0001$). Furthermore, the two mass reared strains also differed significantly in their ability to endure starvation. The strain from Guatemala (SIT1) died significantly faster than the strain from Hawaii (SIT2) (SIT1-SIT2, log-rank test: $\chi^2 = 559.53$, df = 1, $P < 0.0001$).

Discussion

The difference in survival, in the absence of food, between the two mass-reared strains may be ascribed to strain differences, rearing regimes, or most probably, stresses incurred en route from Guatemala to Israel. Both these strains were inferior to wild males who invariably survived longer than sterile males of the corresponding diet group. Why, then, are sterile males less robust? The greater survivorship of the wild strain under starvation may partly be due to selection for faster development of the mass-reared strains. Furthermore, because both wild and sterile flies enjoyed

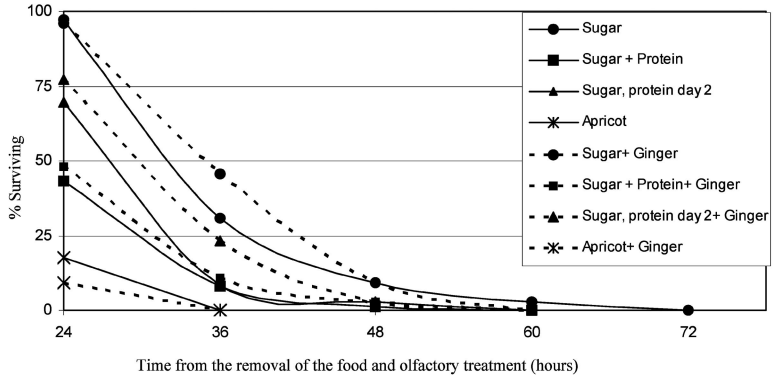

Fig. 2. Survival duration of sterile male Mediterranean fruit flies from the Vienna 7/tol-2000 strain, maintained on different olfactory and dietary regimes after removal of food. Pooled results from three replicates.

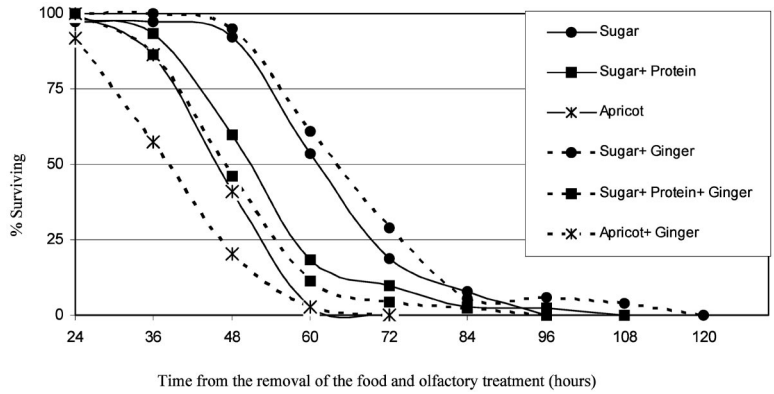

Fig. 3. Survival duration of sterile male Mediterranean fruit flies from the Vienna 4/tol-99 strain, maintained on different olfactory and dietary regimes after removal of food. Pooled results from three replicates.

the same diets before starvation was enforced, it is possible that sterile flies ingest less food, are less adept at assimilating the food they do ingest, or have a higher metabolic rate. Although no quantitative data on food ingestion were collected in the current study, the sterile flies showed no apparent aversion to the food we provided; they seemed to spend as much of their time feeding as did the wild flies. There are several lines of evidence that suggest that assimilation of food may be the key factor. Studies on the intestinal bacterial flora of Mediterranean fruit flies by Lauzon and colleagues indicate that the gut of sterile flies is incapable of sustaining the normal bacterial flora (Carol Lauzon, personal communication; Peloquin et al. 2002). Thus, the approach of inoculating sterile flies with beneficial bacteria may improve their ability to assimilate their food and make them more robust.

Within experimental groups, diet effects on survival showed a consistent pattern. The sugar diet, alone or in combination with α-copaene, was associated with the highest ability to resist starvation, followed closely by the sugar + protein diet (again, alone or in combination with α-copaene).

Paradoxically, the apricot diet, which in some trials was associated with the best sexual performance (Maor 2004), contributed to the rapid demise of the flies ingesting it. Furthermore, when the apricot diet was coupled with α-copaene, the effect was further exacerbated. Although one might conclude that sugar alone is the best diet for sterile males, several recent findings temper the negative implications of our results. First, diet does not affect foraging ability of sterile males (Barry et al. 2003, Maor et al. 2004). Furthermore, due to their inability to inseminate as they age, the sexual effectiveness of sterile males is negligible after the first 24 h after release (Taylor et al. 2001). Thus, it may be better to release relatively short-lived flies that are highly competitive, rather than long-lived, sexually ineffective ones.

Table 1. Effect of post teneral diet and olfactory regime on subsequent ability to withstand starvation by wild male Mediterranean fruit flies, sterile males from the Vienna 7/Tol-2000 strain (SIT1), and sterile males from the Vienna 4/Tol-99 strain (SIT2)

Diet + olfactory environment	Avg longevity [h (SD)]		
	Wild	1SIT	2SIT
Sugar	100.8a (30.6)	42a (11.8)	68a (14)
Sugar + protein	89.8b (24.2)	31.3c (7.6)	58b (14.1)
Sugar, protein day 2	96ab (25.6)	36.2b (10.7)	
Apricot	56.6c (14.8)	29.8c (7.8)	51.6c (8.9)
Sugar + GRO	97.9a (37.3)	40.5a (7.9)	71.5a (14.2)
Sugar + protein+ GRO	100.8a (25.4)	31.2c (8.4)	53.8c (12.1)
Sugar, protein day 2+ GRO	91.6b (26.5)	34.1b (7.7)	
Apricot + GRO	40.4d (11.3)	25.8d (4.3)	44.7d (11.4)

Pooled results from three replicates (n = 72–76). Within columns, means followed by different letters are significantly different (log-rank test).

The effect of diet on the ability to withstand starvation may be related to irreversible metabolic processes. Carey et al. (1999, 2002) have studied the interactions between nutrition and longevity in laboratory populations of the Mediterranean fruit fly. They noted that rich diets induce a metabolic cascade in females that hastens reproduction, as well as death. We suggest that the protein-rich diet we provided to males commits them metabolically to reproduction by diverting resources to pheromone and accessory glands and energy to sexual advertisement. This commitment carries higher sexual rewards but also the penalty of being incapable of weathering periods of nutritional stress.

In conclusion, we have shown that varying the post-teneral environment of male Mediterranean fruit flies affects their ability to withstand starvation. Devising the optimal prerelease environment for sterile males must seek a balance between sexual performance and robustness in a competitive environment.

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